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An allosteric interaction links USP7 to deubiquitination and chromatin targeting of UHRF1

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Abstract

The protein stability and chromatin functions of UHRF1 (Ubiquitin-like, containing PHD and RING Finger domains, 1) are regulated in a cell cycle-dependent manner. We report a structural characterization of the complex between UHRF1 and the deubiquitinase USP7. The first two UBL domains of USP7 bind to the polybasic region (PBR) of UHRF1, and this interaction is required for the USP7-mediated deubiquitination of UHRF1. Importantly, we find that the USP7-binding site of UHRF1 PBR overlaps with the region engaging an intramolecular interaction with the N-terminal tandem Tudor domain (TTD). We show that the USP7-UHRF1 interaction perturbs the TTD-PBR interaction of UHRF1, thereby shifting the conformation of UHRF1 from a TTD-"occluded" state to a state open for multivalent histone binding. Consistently, introduction of an USP7-interaction defective mutation to UHRF1 significantly reduces its chromatin association. Together, these results link USP7 interaction to the dynamic deubiquitination and chromatin association of UHRF1.

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Author contributions

Z.Z., S.B.R., D.A., J.H., Q.C., L.L., and J.S. performed the experiments. J.S., G.G.W., B.D.S., and Y.W. conceived the project. J.S. wrote the manuscript with input of all authors.

Accession numbers

The structure of UHRF1-USP7 has been deposited under accession number 5C6D in the Protein Data Bank.

Introduction

One of the fundamental principles in epigenetic regulation involves temporal and spatial control of macromolecular machineries that govern epigenetic events. Epigenetic modifications, including DNA methylation and histone modifications, are recognized by a diverse family of effector proteins (Bogdanovic and Veenstra, 2009; Musselman et al., 2012) whose functions are often subject to dynamic regulation in response to environmental cues. However, how these chromatin effector proteins are regulated remains incompletely understood.

UHRF1 (Ubiquitin-like, containing PHD and RING Finger domains, 1), also known as ICBP90 and NP95 in mouse, is a multi-domain protein that plays critical roles in regulating various processes, such as DNMT1 (DNA methyltransferase 1)-mediated DNA methylation maintenance (Bostick et al., 2007; Sharif et al., 2007). UHRF1 contains a ubiquitin-like domain (UBL) at the N-terminus, followed by a tandem Tudor domain (TTD), a plant homeodomain (PHD), a SET and RING associated (SRA) domain and a really interesting new gene (RING) domain (Fig. 1A). Among these protein modules, the PHD and TTD domains recognize the N-terminal tail of histone H3 unmethylated at arginine 2 (Hu et al., 2011; Lallous et al., 2011; Rajakumara et al., 2011) and dimethylated/trimethylated at lysine 9 (H3K9me2/3) (Arita et al., 2012; Cheng et al., 2013; Karagianni et al., 2008; Rothbart et al., 2013; Rothbart et al., 2012; Xie et al., 2012), respectively, while the SRA domain specifically binds to hemimethylated CpG sites (Arita et al., 2008; Avvakumov et al., 2008; Bostick et al., 2007; Hashimoto et al., 2008; Sharif et al., 2007). The RING domain of UHRF1 has been shown to serve as an E3 ubiquitin ligase to promote monoubiquitination of H3 at lysine 18 and/or 23 (Nishiyama et al., 2013; Qin et al., 2015), which in turn recruits DNMT1 to replicating chromatin.

Despite these advances, the mechanism underlying the regulation of UHRF1 remains to be elucidated. Indeed, emerging evidence has shown that UHRF1 is subject to temporal and spatial control (Gelato et al., 2014; Ma et al., 2012). First, the stability of UHRF1 is regulated by ubiquitination in a cell cycle-dependent manner (Ma et al., 2012). During S-phase, deubiquitinase USP7 (ubiquitin-specific-processing protease 7) associates with UHRF1 keeping it from ubiquitination (Ma et al., 2012). When cells enter mitosis, USP7 dissociates from UHRF1, exposing it for ubiquitination and consequent proteasomal degradation (Ma et al., 2012). Second, the chromatin association of UHRF1 peaks in mid S-phase to accompany pericentromeric heterochromatin replication (Miura et al., 2001; Papait et al., 2007; Taylor et al., 2013), which is likely mediated by a conformational transition between two alternative functional states of UHRF1 (Gelato et al., 2014). In one state, the H3K9me3-binding site of the UHRF1 TTD domain is occluded due to its intramolecular interaction with a C-terminal polybasic region (PBR). In the second state, the TTD domain is relieved from the PBR interaction and thus forms a multivalent histone binding cassette with the PHD domain (Gelato et al., 2014). Binding of the signaling molecule phosphatidylinositol 5-phosphate (PI5P) to the UHRF1 PBR switches the UHRF1 conformation from the TTD-“occluded” state to the TTD-PHD multivalent state, which leads to its increased heterochromatin association (Gelato et al., 2014). However, how networks of interactions functionally regulate UHRF1 in cells remains unresolved.

In this study, we characterized the interaction between UHRF1 and USP7 by structure determination of the UHRF1-USP7 complex. Our structural analyses reveal that the UHRF1-USP7 interaction is mediated by the first two UBL domains of USP7 and the PBR of UHRF1. Through functional assays, we show that the interaction of UHRF1 with USP7 is not only critical for its deubiquitination but also disrupts the intramolecular TTD-PBR interaction, thereby shifting the conformation of UHRF1 to promote multivalent histone engagement of the TTD-PHD cassette. Indeed, point mutations that disrupt the USP7 interaction greatly reduce the chromatin association of UHRF1. Our study thus uncovers a novel mechanism by which USP7 promotes both the stability and chromatin association of UHRF1.

Results

Characterization of the UHRF1-USP7 interaction

A recent study suggests that the interaction between USP7 and UHRF1 is mediated by the Ubiquitin like (UBL) domains of USP7 and a linker region between the SRA and RING finger domains of UHRF1 (residues 600-687, $UHRF1_{600-687}$) (Fig. 1A) (Ma et al., 2012). To further map the USP7 and UHRF1 interaction interface, we performed isothermal titration calorimetry (ITC) to measure bindings between truncated USP7 and UHRF1 fragments. The first two UBL domains (UBL_{1-2}) of USP7 interact directly with a fragment of UHRF1 encompassing its SRA and RING domains (residues 419-806, $UHRF1_{SRA-RING}$), with a dissociation constant (K_d) of 1.30 μM (Fig. 1B). To narrow this interaction surface further, we showed that the UBL_{1-2} dual domain of USP7 binds to $UHRF1_{600-687}$ with a K_d of 1.42 μM (Fig. S1A), and comparable binding activity is observed (K_d = 1.52 μM) when we truncated UHRF1 further to a 32-amino-acid sequence, $UHRF1_{647-678}$ (Fig. 1B). By contrast, we did not observe appreciable interaction between the first UBL domain (UBL_1) of USP7 and $UHRF1_{SRA-RING}$ (Fig. S1B), indicating that UBL_1 alone is insufficient for mediating the UHRF1-USP7 interaction. Collectively, these data show that the $UHRF1_{647-678}$ fragment and the USP7 UBL_{1-2} domains mediate the UHRF1-USP7interaction.

Structure of the $UHRF1_{647-678}$-USP7 UBL_{1-3} complex

Next, to provide the molecular basis for the UHRF1-USP7 interaction, we determined the crystal structure of $UHRF1_{647-678}$ in complex with the first three UBL domains (UBL_{1-3}) of USP7 at 2.3 Å resolution (Table S1). It is notable that there are two UHRF1-USP7 complexes in each asymmetric unit (Fig. S1C), and in both complexes the UHRF1 fragments superimpose well, except for one region (residues S652-T655) that is only observable in one of the complexes due to crystal packing (Fig. S1D). The UHRF1 segment containing residues G656-R662 is anchored to an acidic surface formed by the closely packed UBL_1 and UBL_2 domains of USP7, whereas no intermolecular interaction involves the USP7 UBL_3 domain (Fig. 2A, B). The interactions between the USP7 UBL_{1-2} domains and the $UHRF1_{647-678}$ peptide are mediated by hydrogen bonds and electrostatic attractions (Fig. 2C). Of particular note, the side chain of UHRF1 K659 is inserted into an acidic pocket of USP7, formed by residues R628, E736, D764, E759 and M761, with the K659 ε-amino group hydrogen bonded to the side chain carboxylates of USP7 E736 and D764, and to the

backbone carbonyl oxygen atoms of USP7 D758 and E759 (Fig. 2C, D). In a parallel configuration, the ε-amino group of UHRF1 K657 forms direct hydrogen bonds with the side chain carboxylates of USP7 E744 and D758, and a water-mediated hydrogen bond with the backbone carbonyl oxygen of USP7 D758 (Fig. 2C, D). Meanwhile, the backbone amide groups of UHRF1 K657 and K659 each form a hydrogen bond with the side chain carboxylate of USP7 E759 (Fig. 2C, D). The backbone carbonyl oxygen of UHRF1 K657 forms a hydrogen bond with the side chain amide of USP7 N630 (Fig. 2C). C-terminal to UHRF1 K657 and K659, the ε-amino group of UHRF1 K661 forms a hydrogen bond with the side chain carboxylate of USP7 D653 (Fig. 2C). In addition, three main chain hydrogen bonds, formed between UHRF1 G658, W660 and R662, and USP7 N630, S629 and A627, respectively, further support the UHRF1-USP7 interaction (Fig. 2C).

Mutational analysis of the UHRF1-USP7 interaction

To test our structural observations, we mutated a number of key interacting residues for ITC (Table S2). In comparison with the wild-type (WT) $UHRF1_{647-687}$-USP7 UBL_{1-2} interaction, UHRF1 K659E and USP7 E736A mutations reduced the binding affinity by 38- and 4-fold, respectively (Fig. 3A). Furthermore, the UHRF1 K657Q/K659Q and K657E/K659E double mutations both reduced the binding affinity by over 200-fold (Fig. 3A). Together, these data support our structural observations. Consistently, sequence alignment of UHRF1 protein orthologues revealed that except for K657 that is not present in NP95, the USP7-interacting residues, including UHRF1 G658, K659 and K661, are highly conserved across species (Fig. 3B), suggesting an important role for these residues in UHRF1 regulation.

To test whether UHRF1 K657 and K659 are important for association of full-length UHRF1 to USP7, we ectopically expressed HA-tagged USP7 in HEK293 cells in the presence or absence of Flag-tagged UHRF1 (WT or K657Q/K659Q mutant), followed by co-immunoprecipitation (CoIP) with an anti-Flag antibody (Fig. 3C). We found that USP7 co-precipitates with WT UHRF1, confirming that these proteins form a complex in cells (Fig. 3C). As predicted from the structural data, the UHRF1 K657Q/K659Q double mutant reduced the association of UHRF1 with USP7, further supporting a critical role for these residues in mediating the UHRF1-USP7 interaction.

Effect of the UHRF1-USP7 interaction on USP7-mediated deubiquitination of UHRF1

To investigate the functional consequence of the UHRF1-USP7 interaction, we next performed *in vitro* ubiquitination assays, monitoring autoubiquitination of wild-type UHRF1 or the K659E mutant in the presence of full-length USP7. Consistent with previous observations (Felle et al., 2011; Ma et al., 2012), our results showed that USP7 reduced autoubiquitination activity of wild-type UHRF1 (Fig. 3D). In contrast, USP7 cannot reduce the autoubiquitination of the UHRF1 K659E mutant. These data confirm previous observations that the USP7-UHRF1 interaction is required for USP7-mediated deubiquitination of UHRF1(Felle et al., 2011; Ma et al., 2012).

Effect of the UHRF1-USP7 interaction on the allosteric regulation of UHRF1

A recent study (Gelato et al., 2014) showed that an intramolecular TTD-PBR interaction of UHRF1 occludes its TTD domain from binding to H3K9me3. Given the fact that the USP7 binding site of UHRF1 from this study is located within the UHRF1 PBR sequence (Fig. 4A) (Gelato et al., 2014), we postulated that the UHRF1-USP7 interaction might interfere with the UHRF1 TTD-PBR interaction. To test this hypothesis, we performed NMR titrations to investigate whether the UHRF1 TTD-PBR interaction is affected by the USP7 UBL_{1-2} dual domain. A number of $^{1}H,^{15}N$-HSQC spectra were collected for the ^{15}N-labeled UHRF1 TTD domain, alone, or in the presence of UHRF1 PBR ($UHRF1_{647-687}$) and/or the USP7 UBL_{1-2} domains (Fig. 4B-D and Fig. S2A). Consistent with a previous study (Gelato et al., 2014), we observed that a number of NMR peaks of the UHRF1 TTD domain were significantly shifted upon addition of $UHRF1_{647-687}$ with 20% molar excess (Fig. 4B-D and Fig. S2A). Based on the reported chemical shift assignment of the TTD (Nady et al., 2011), we were able to assign a number of NMR peaks with large chemical shift changes to UHRF1 residues, including I224, G275 and V287. It is notable that the chemical shift perturbations of these residues by the UHRF1 PBR were also observed in a previous study (Gelato et al., 2014), and that residues I224 and V287 were conceived as part of the putative PBR-binding pocket of the TTD domain (Gelato et al., 2014). However, when we further mixed the NMR sample with the USP7 UBL_{1-2} dual domain (40% molar excess over UHRF1 TTD), the majority of PBR-perturbed TTD peaks shifted back toward the peak positions corresponding to the free state (Fig. 4B-D and Fig. S2A). These data suggest that the USP7 UBL_{1-2} dual domain affects the intramolecular TTD-PBR interaction of UHRF1 through direct interaction with the UHRF1 PBR. Consistently, we found that the presence of USP7 UBL_{1-5} (residues 560-1084) modestly increased the binding of full-length UHRF1 to the H3(1-20)K9me3 peptide by a pull-down assay, confirming that the USP7 interaction enhances the TTD-H3K9me3 binding in the context of full-length UHRF1 (Fig. S2B).

We then performed ITC for the USP7 UBL_{1-2} dual domain over a UHRF1 fragment spanning from the TTD domain toward the C-terminal RING domain (residues 138-806, $UHRF1_{TTD-RING}$) to further examine whether an intramolecular UHRF1 TTD-PBR interaction affects the UHRF1-USP7 interaction. Our results showed that the USP7 UBL_{1-2} dual domain binds to $UHRF1_{TTD-RING}$ with a K_d of 27.2 μM (Fig. 4E), a binding affinity that is ~20-fold weaker in comparison with the binding of the USP7 UBL_{1-2} to $UHRF1_{SRA-RING}$ (Fig. 4E). This result suggests that the presence of UHRF1 TTD conversely disfavors the UHRF1-USP7 interaction.

Taken together, these findings establish that the UHRF1-USP7 interaction disrupts the intramolecular TTD-PBR interaction of UHRF1, thereby shifting the conformation of UHRF1 TTD from the occluded state to an open state that allows multivalent histone binding by the TTD-PHD domains.

Effect of the USP7-UHRF1 interaction on the chromatin association of UHRF1

The observation that the USP7 interaction modulates the conformational states and H3K9me3 binding of UHRF1 prompted us to investigate whether the UHRF1-USP7 interaction affects chromatin association of UHRF1. Toward this end, we tested recombinant

full-length UHRF1 and a USP7 binding mutant (K657E/K659E) in histone peptide binding assays by fluorescence polarization (Fig. S3A). Consistent with our previous findings (Rothbart et al., 2013), both full-length wild-type and K657E/K659E UHRF1 were found to bind to $H3_{(1-20)}$K9me3 peptides conjugated with 5-FAM at the C-terminus similarly. By contrast, neither protein had measurable interaction with $H3_{(1-20)}$K9me3 peptides conjugated with 5-FAM at the N-terminus, the peptides that were previously shown to block the UHRF1 PHD-histone binding (Rothbart et al., 2013). This data, in line with a recent study (Gelato et al., 2014) that the K657E/K659E mutant alone does not affect the UHRF1 TTD-PBR interaction appreciably, suggests that the previously described multivalent histone engagement of the TTD-PHD (Rothbart et al., 2013) remains for full-length UHRF1 and its K657E/K659E mutant. Furthermore, we showed that unlike wild-type UHRF1, the UHRF1 K657E/K659E mutant dramatically decreases its association with chromatin isolated from HeLa cells (Fig. 4F and Fig. S3B). Collectively, these observations suggest that the USP7 interaction facilitates the association of UHRF1 with chromatin.

Discussion

Cell cycle-dependent USP7 regulation of UHRF1

UHRF1 has emerged as an important epigenetic regulator in the maintenance of DNA methylation through regulating the recruitment and stability of DNMT1 (Bostick et al., 2007; Du et al., 2010; Sharif et al., 2007). This regulation is achieved, in part, through dynamic regulation of UHRF1 – both at the level of its chromatin association and ubiquitination – throughout the cell cycle (Gelato et al., 2014; Ma et al., 2012; Taylor et al., 2013). In this study, we have confirmed the requirement of USP7 interaction for UHRF1 deubiquitination through *in vitro* ubiquitination assay. More importantly, we uncovered the UHRF1 PBR, a region known to be crucial for modulating an inter-conversion between the two alternative functional states of UHRF1 (Gelato et al., 2014), as the region responsible for mediating its interaction with USP7. The interaction between USP7 and UHRF1 PBR therefore may help release the TTD domain from the PBR-bound state, entering into a state that allows TTD-PHD multivalent histone engagement, as characterized previously (Rothbart et al., 2013). Indeed, with a series of structural, biochemical and mutagenesis assays, we demonstrated that the USP7 interaction with the UHRF1 PBR sequence promotes the H3K9me3 binding by UHRF1 through interfering with its intramolecular TTD-PBR interaction; consistently, disruption of the UHRF1-USP7 interactions leads to a significantly decreased chromatin association of UHRF1 *in vivo*. Taken together, this study reveals that the USP7 interaction plays a dual regulatory role in both deubiquitination and chromatin association of UHRF1 (Fig. 4G).

Effects of post-translational modifications on the UHRF1-USP7 association

A previous study suggested that phosphorylation of UHRF1 S652 regulates the dissociation of the UHRF1-USP7 complex in M-phase (Ma et al., 2012). Interestingly, our study indicates that this residue is indeed adjacent to the USP7 interaction site. However, we did not observe any direct interaction between UHRF1 S652 and USP7. Moreover, our ITC analysis revealed that phosphorylation of UHRF1 S652 only leads to a modest (2-fold) reduction of the binding affinity of $UHRF1_{651-664}$ for USP7 (Fig. S4A). Meanwhile, the

serine-to-glutamate mutation of UHRF1 S664 (S664E), which is located immediately downstream of the USP7 interaction site (Fig. 4A) and manifests M-phase specific phosphorylation as well (Rigbolt et al., 2011), leads to a reduction of the $UHRF1_{647-687}$ binding affinity for USP7 by 4-fold (Fig. S4B). Together, these observations suggest that multi-step post-translational modification events may be required to dissociate the UHRF1-USP7 complex during the M-phase.

Recent evidence has indicated that USP7 also deubiquitinates DNMT1 during S-phase; in late S-phase, acetylation of DNMT1 by Tip60 leads to disruption of the DNMT1-USP7 complex (Du et al., 2010). This previous work also prompted us to query if UHRF1 can be acetylated by Tip60 at PBR, which falls into the Tip60-preferred acetylation sequences (Kimura and Horikoshi, 1998). Indeed, following *in vitro* acetylation by recombinant Tip60, we detected acetylation of UHRF1 at K659 (Fig. S4C) and found that this modification greatly decreased the UHRF1-USP7 interaction (Fig. S4A), indicating a mechanism for UHRF1-USP7 dissociation. However, further studies are needed to define such a potential regulation of UHRF1 *in vivo*.

USP7 interaction and chromatin targeting of UHRF1

One of the major findings in this study is that the USP7 interaction regulates the chromatin association of UHRF1. Consistent with this, a recent study showed that the UHRF1 S664E mutation, with a 4-fold reduction in USP7 binding based on our ITC analysis (Fig. S4B), significantly reduced the chromatin binding of UHRF1 (Chu et al., 2012). Whereas the underlying mechanism of this regulation has yet to be elucidated, we show that the UHRF1-USP7 interaction interferes with the intramolecular TTD-PBR interaction of UHRF1, which therefore shifts the conformation of UHRF1 from the TTD-“occluded” state to a state that allows multivalent histone binding by the TTD-PHD domains. Such a change in the conformational dynamics of UHRF1 may synergize with the allosteric effect of PI5P (Gelato et al., 2014) in stabilizing the chromatin association of UHRF1. Indeed, a previous study showed that the nuclear level of PI5P is cell cycle-dependent, with ~20-fold enrichment in G1 phase (Clarke et al., 2001), However, the relationship between PI5P and the USP7-UHRF1 interaction remains to be determined. In addition, complex formation between UHRF1 and USP7 may also boost their respective interactions with methyl CpG binding protein MBD4 (Meng et al., 2015), which has recently been shown to mediate the recruitment of USP7 to heterochromatin (Meng et al., 2015).

Materials and Methods

USP7 and UHRF1 proteins, except for the $UHRF1_{651-664}$ peptides, were expressed in BL21 (DE3) RIL cells and purified through Nickel affinity, size exclusion and/or anion exchange chromatography. The crystal structure of the $UHRF1_{647-678}$-USP7 UBL_{1-3} complex was determined by Molecular Replacement using the structure of the corresponding sequence of free USP7 (PDB 2YLM) as search model. ITC assays for USP7 and UHRF1 proteins were performed at 4°C. The resultant binding curves were processed with software ORIGEN 7.0 (MicroCal). Chromatin association assays were performed from HeLa cells stably transfected with 3xFlag UHRF1 (wild-type and K657E/K659E) as previously described

(Rothbart et al., 2013). Full details of experimental procedures are provided in the Supplementary Information.

Supplementary Material

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Highlights

1. USP7 ubiquitin-like domains (UBL) bind to UHRF1 polybasic region (PBR)
2. USP7 interaction promotes USP7-mediated deubiquitination of UHRF1
3. USP7 allosterically regulates the conformational states of UHRF1
4. USP7 interaction affects the chromatin association of UHRF1

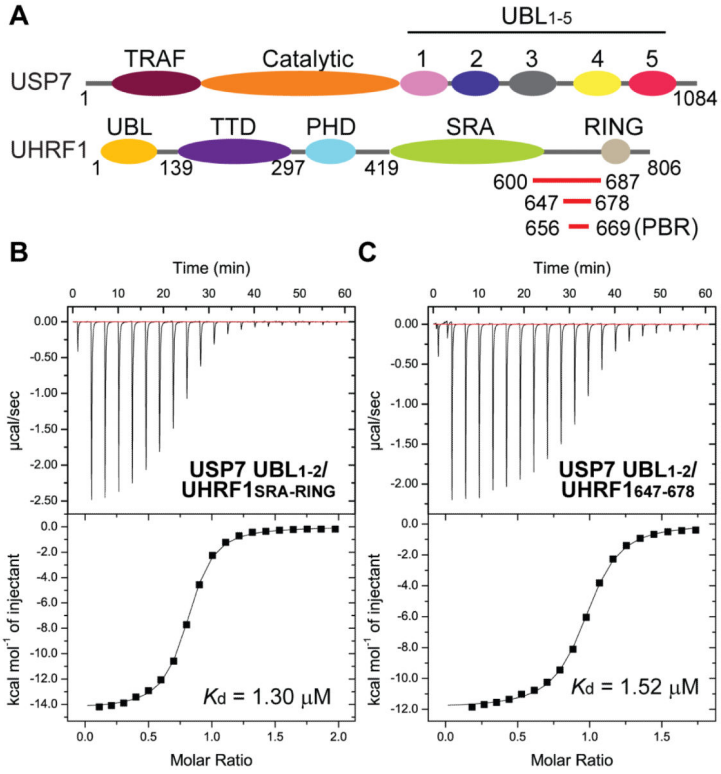

Figure 1. Identification of the UHRF1 and USP7 interaction domains

(**A**) Domain architectures of UHRF1 and USP7. The UHRF1 fragments used for biochemical analysis are labeled with residue numbers. (**B, C**) ITC binding curves for USP7 UBL_{1-2} over $UHRF1_{SRA-RING}$ (**B**)and $UHRF1_{647-678}$ (**C**). See also Figure S1.

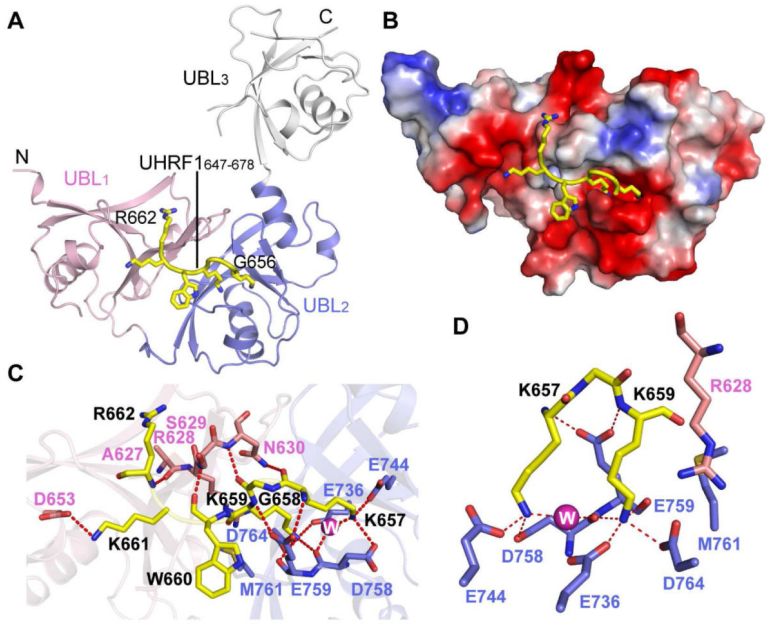

Figure 2. Structural basis of the UHRF1$_{647-678}$-USP7 UBL$_{1-3}$ complex

(**A**) Ribbon representation of the UHRF1$_{647-678}$-USP7 UBL$_{1-3}$ complex, with USP7 UBL1, 2, and 3 colored silver, light blue and pink, respectively. The UHRF1$_{647-678}$ fragment is shown in stick representation. (**B**) Electrostatic surface representation of the UHRF1$_{647-678}$-UHRF1 UBL$_{1-3}$ complex. For clarity, the USP7 UBL3 domain was not shown. (**C**) Hydrogen bonding interactions (dashed lines) between USP7 and UHRF1. The water molecule is shown in sphere, labeled with "W". (**D**) Close-up view of the UHRF1 K657 and K659-associated intermolecular interactions. See also Table S1.

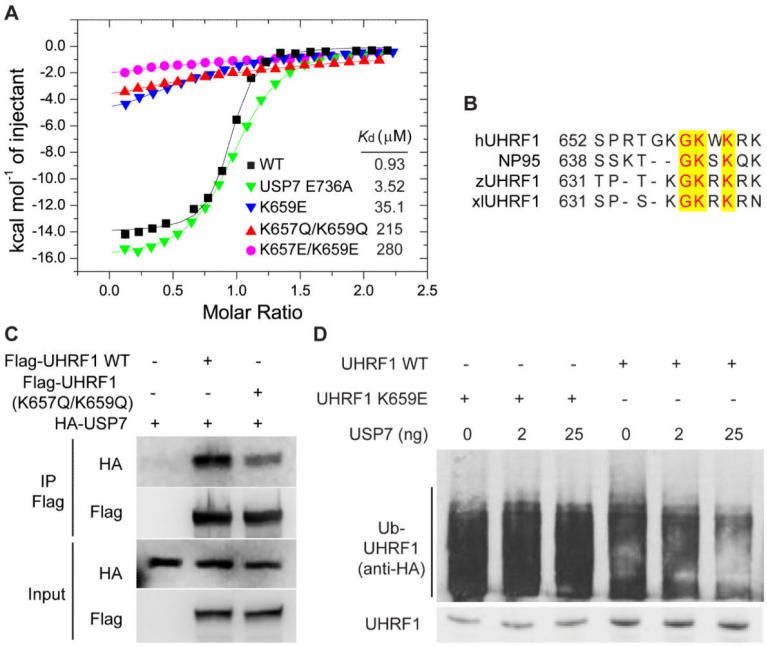

Figure 3. Biochemical analysis of the UHRF1-USP7 interaction

(**A**) ITC and mutational analysis of the $UHRF1_{647-687}$-USP7 UBL_{1-2} interaction. The mutations include USP7 E736A, UHRF1 K659E, K657Q/K659Q and K657E/K659E. (**B**) Sequence alignment of the USP7-binding motif of human UHRF1 with its mouse (NP95), zebra fish (zUHRF1) and *Xenopus Laevis* (xlUHRF1) orthologues. Identical residues are colored red and highlighted in yellow. (**C**) Immunoprecipitation analysis of the interaction between full-length Flag-tagged UHRF1 and HA-tagged USP7. (**D**) *In vitro* ubiquitination assay of wild-type and K659E UHRF1 in the presence of HA-tagged ubiquitin and USP7. The ubiquitination level (upper panel) and the total amount of UHRF1 (lower panel) were analyzed by anti-HA Western blot and SYPRO staining, respectively. See also Figure S4 and Table S2.

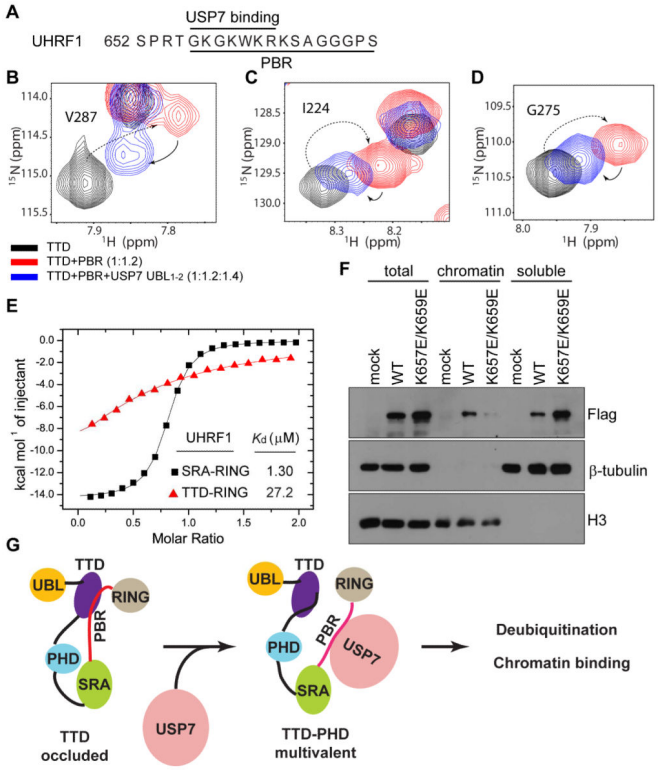

Figure 4. The UHRF1-USP7 interaction affects the UHRF1 intramolecular regulation and chromatin association

(**A**) Sequence of a selected UHRF1 fragment, with USP7 binding site and PBR sequence labeled. (**B-D**) Overlaid 2D $^{1}H,^{15}N$-HSQC spectra highlight the chemical shift changes of selected residues in UHRF1 TTD. The spectra were collected for UHRF1 TTD free (black), in the presence of UHRF1 PBR (red) or UHRF1 PBR and USP7 UBL_{1-2} (blue). The arrows with dashed and solid lines mark the chemical shift changes of selected residues under different conditions. (**E**) ITC binding curves for USP7 UBL_{1-2} over $UHRF1_{SRA-RING}$ (black) or $UHRF1_{TTD-RING}$ (red). (**F**) Chromatin association assay of flag-tagged wild-type (WT) and K657E/K659E UHRF1 from asynchronously growing HeLa cells. Mock indicates no DNA control. (**G**) Model for USP7 regulation. The USP7 interaction allosterically regulates the conformational states of UHRF1 and affects its ubiquitination and chromatin binding. See also Figure S2 and S3.